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Evaluation of Methods for the Estimation of 5-Aminolevulinate Dehydratase for a Broad Range of Lead Concentrations in the Blood of Exposed Workers

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Summary: 5-aminolevulinate dehydratase activity was estimated in the blood of 30 lead-exposed workers and 15 control persons, using (I) the method of *Tomokuni* ((1974) Arch. Environm. Health *29*, 274–281) and (II) the European standard method (*Berlin & Schaller* (1974) Z. Klin. Chem. Klin. Biochem. *12*, 389–390). The lead level in the blood was in the range 0–11.6 μmol/l. It was found that the correlation coefficients between the activity of 5-aminolevulinate dehydratase and the lead level in blood (up to 4.35 μmol/l) are higher for method I than method II. These two methods only give similar results for the units of 5-aminolevulinate dehydratase activity when the lead concentrations in the blood are low. For higher concentrations of this metal (up to 11.6 μmol/l), a high correlation was obtained ($r = -0.80$) between the 5-aminolevulinate dehydratase activity ratio (enzyme measured at pH 6.8/enzyme measured at pH 6.0; method I) and lead concentrations in the blood.

Bewertung von Methoden zur Bestimmung von 5-Aminolävulinatdehydratase bei einem breiten Bereich der Bleikonzentrationen im Blut exponierter Arbeiter

Zusammenfassung: Die katalytische Aktivität von 5-Aminolävulinatdehydratase im Blut wurde mit der Methode (I) von *Tomokuni* ((1974) Arch. Environm. Health *29*, 274–281) und (II) der Europäischen Standardmethode nach *Berlin & Schaller* ((1974) Z. Klin. Chem. Klin. Biochem. *12*, 389–390) bei 30 Blei-exponierten Arbeitern und 15 Kontrollpersonen bestimmt. Die Bleikonzentrationen betrugen 0–11,6 μmol/l. Die Korrelationskoeffizienten zwischen 5-Aminolävulinatdehydratase und Bleikonzentration im Blut (bis zu 4,35 μmol/l) sind für Methode I höher. Nur für niedrige Bleikonzentrationen im Blut können die mit diesen beiden Methoden ermittelten Werte für 5-Aminolävulinatdehydratase gegeneinander ausgetauscht werden. Für höhere Bleikonzentrationen (bis zu 11,6 μmol/l) wurde eine hohe Korrelation ($r = 0,80$) zwischen dem 5-Aminolävulinatdehydratase-Verhältnis (Messung bei pH 6,8/Messung bei pH 6,0; Methode I) und der Bleikonzentration im Blut erhalten.

Introduction

With the exception of zinc protoporphyrin, the activity of 5-aminolevulinate dehydratase (EC 4.2.1.24) presently constitutes one of the most sensitive indices of environmental and occupational exposure to lead (1–11).

The method of estimation of 5-aminolevulinate dehydratase activity, proposed by *Gibson* et al. (12) has been modified by *Bonsingore* et al. (13) and by *Haas* et al. (1).

Tomokuni (14) has also modified this method, introducing a new test, i.e. the determination of the 5-aminolevulinate dehydratase activity ratio, based on measurement of the enzyme activity at different pH's. Lead exposure brings about a change in the pH optimum for blood 5-aminolevulinate dehydratase, optimal pH values being equal to 6.0 and 6.8 for occupationally exposed and control persons, respectively.

In 1974, *Berlin & Schaller* (15) also elaborated a method, which was proposed as the European standard. In describing their analytical procedure, these authors presented no data on the range of activity of 5-aminolevulinate dehydratase in persons exposed to lead, and its dependence on lead concentration in blood; such data were, however, presented by *Tomokuni* (14). The two methods use different units: porphobilinogen [μmol/h · ml erythrocytes] in the *Tomokuni* method, and 5-aminolevulinate [μmol/min · l erythrocytes] in the *Berlin & Schaller* method. This creates difficulties

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in the comparison of results from different laboratories (9).

The aim of this paper was to analyse the results for the determination of 5-aminolevulinate dehydratase activity by the method of *Tomokuni* (14) and by the standard European method according to *Berlin & Schaller* (15) in the blood of persons occupationally exposed to lead, and for a broad range of blood lead concentrations.

Materials and Methods

30 workers exposed occupationally to lead and 15 control persons were studied. The activity of 5-aminolevulinate dehydratase was estimated in all these persons by two methods: (I) according to *Tomokuni* (14) – and (II) according to *Berlin & Schaller* (15). The hematocrit (16) and lead level were also estimated. Lead was determined by the dithizone method (17). Dithizone (diphenylthiocarbazone), in combination with lead at pH 9.5, produces a coloured complex of lead dithizone. A sample of mineralized blood (3–5 ml) was added to 5 ml of 250 g/l ammonium citrate solution, then mixed. The pH was adjusted to 9.5 by titrating with ammonium hydroxide. The solution was then added to 5 ml 100 g/l potassium cyanide, together with 1 ml of dithizone solution in chloroform. After shaking for 1 minute the chloroform phase became red-violet. The chloroform phase was adjusted to a volume of 10 ml with pure chloroform. Excess of dithizone was removed by shaking with a solution containing ammonium hydroxide and potassium cyanide. The sample was then filtered through anhydrous sodium sulfate. Absorption was read in a spectrophotometer at 510 nm in 5 cm cuvettes against the blank. A standard curve was prepared using animal blood (0.005 μmol–0.029 μmol of lead in the sample). Precision of this method was equal to ± 9.2%.

Results

The results presented in figure 1 indicate that after 24 h the activity of 5-aminolevulinate dehydratase in stored blood decrease by 37.6 ± 16.8% when estimated by method II. Taking this into account, all estimations of activity of 5-aminolevulinate dehydratase were performed 3–4 h after blood sampling. During this period, blood was stored at + 2 °C.

Figures 2a and 2c show the dependence of activity of 5-aminolevulinate dehydratase estimated by method I (a) and method II (c) on the blood lead concentrations. For both methods, a non-linear dependence was observed troughout the lead concentration range studied. Linear dependencies were found for appropriate intervals of lead concentrations only. Correlation coefficients and regression equations were calculated for two exemplary ranges of lead concentration in blood up to 1.93 μmol/l blood (tab. 1) and up to 4.35 μmol/l blood (fig. 2b and 2d).

The correlation between the activity of 5-aminolevulinate dehydratase, estimated by two methods, and lead concentration in blood, and the corresponding regression equations are presented in figure 3a for the lead concen-

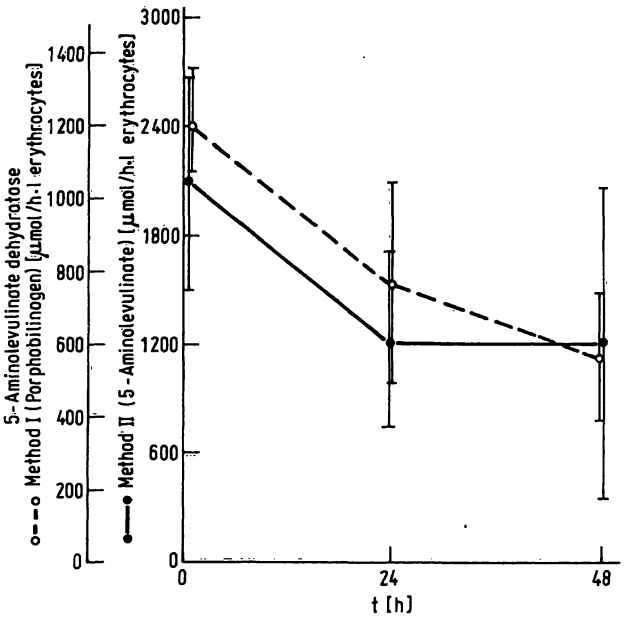

Fig. 1. Relationship between activity of 5-aminolevulinate dehydratase determined by method I (– – – –) and method II (———) and time of blood storage at + 2 °C. Activity curves obtained from five subjects not exposed to lead.

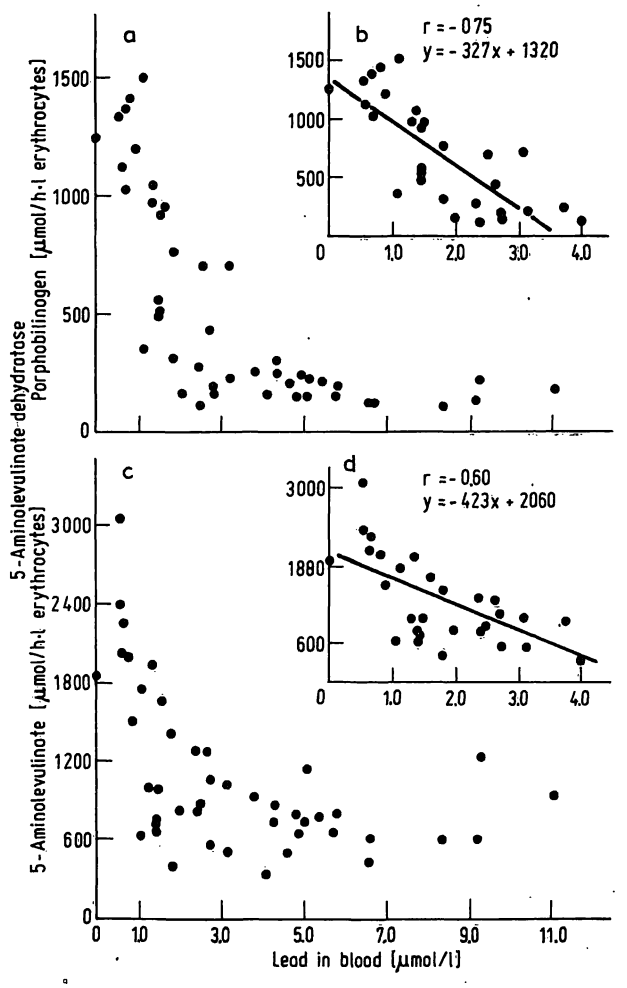

Fig. 2. Correlation between activity of 5-aminolevulinate dehydratase (a, b determined by method I; and c, d determined by method II) and lead level in blood.

Tab. 1. Comparison of correlation coefficients between lead level in blood and erythrocyte activity of 5-aminolevulinate dehydratase and of the 5-aminolevulinate dehydratase activity ratio.

Test	Lead level in blood. Range from zero to µmol/l	Correlation coefficients r	
		method I	method II
Activity of 5-aminolevulinate dehydratase	1.93	− 0.65	− 0.53
	4.35	− 0.75	− 0.60
5-aminolevulinate dehydratase activity ratio[x]	1.93	− 0.10	
	4.35	− 0.53	
	6.76	− 0.79	
	11.60	− 0.80	

x/ Activity ratio $= \frac{\Delta A \text{ at pH } 6.8}{\Delta A \text{ at pH } 6.0}$ (14)

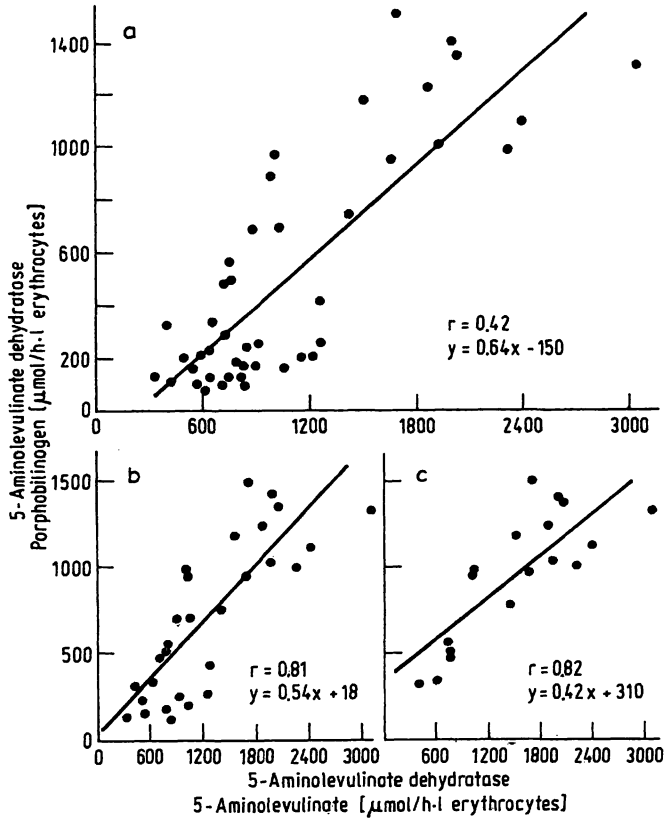

Fig. 3. Correlation between activities of 5-aminolevulinate dehydratase determined by methods I and II for different ranges of lead level in blood: a) up to 11.60; b) 4.35; c) 1.93 µmol/l.

tration range up to 11.60 µmol/l blood (r = 0.42); figure 3b shows the data for the lead concentration range up to 4.35 µmol/l blood (r = 0.81), and figure 3c for the lead concentration range up to 1.93 µmol/l blood (r = 0.82). Table 2 presents recalculated values of the units of activity of 5-aminolevulinate dehydratase from method II for comparison with method I, depending on the blood lead concentration.

Tab. 2. Comparison of methods I and II (activity of 5-aminolevulinate dehydratase is expressed in the same units in relation to lead level in blood)

Lead level in blood [µmol/l]	5-Aminolevulinate dehydratase (Porphobilinogen) [µmol/h · l erythrocytes]		SD
	I	II	
0 −1.93	1 n = 18	1.58 (1.04−2.29)	± 0.37
1.93−4.35	1 n = 11	3.75 (1.24−7.55)	± 1.94

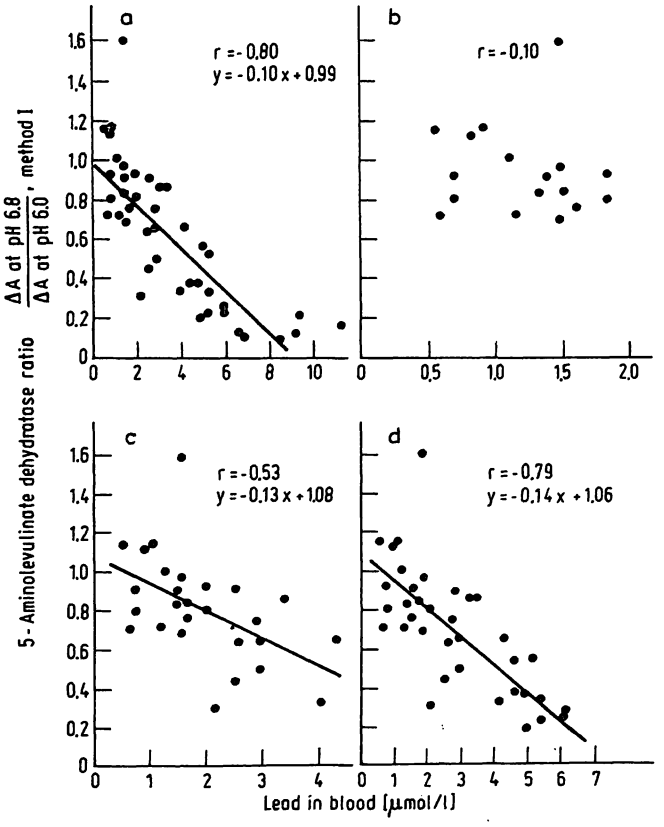

Fig. 4. Relationship between 5-aminolevulinate dehydratase activity ratio (enzyme measured at pH 6.8/enzyme measured at pH 6.0; method I) and lead level in blood: a) up to 11.60; b) up to 1.93; c) up to 4.35; d) up to 7.25 µmol/l.

Figure 4 displays the dependence of the 5-aminolevulinate dehydratase activity ratio on the lead concentration in blood. For low lead concentrations (up to 1.93 µmol/l blood) there is no correlation (fig. 4b). For higher lead concentrations r = 0.53 (fig. 4c); an identical value was obtained by *Tomokuni* (14). High lead concentrations yield high correlation coefficients (fig. 4a and 4d).

Figure 5 shows frequency distribution of activities of 5-aminolevulinate dehydratase measured by methods I and II (fig. 5a and 5b, respectively). For the control group, the activity of 5-aminolevulinate dehydratase estimated by method I ranged from 710 up to 1500

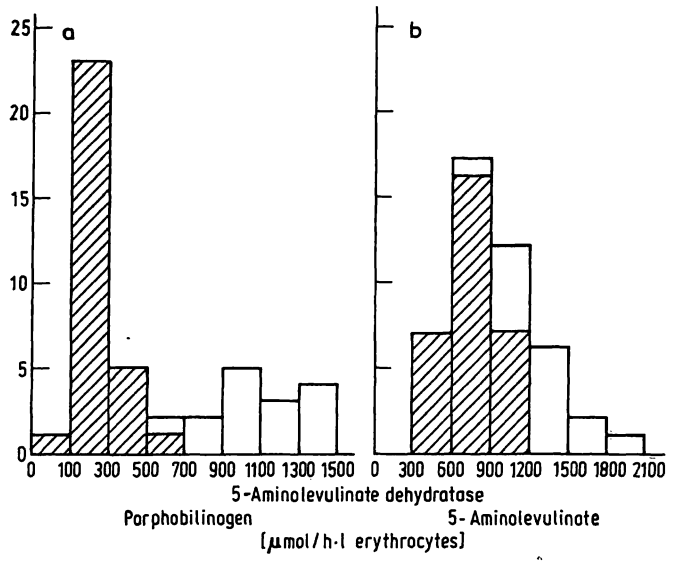

Fig. 5. Frequency distributions of activity of 5-aminolevulinate dehydratase estimated by method I (a) and method II (b). Values obtained for the control group and exposed workers represented by open and shaded areas, respectively.

(mean = 1090 ± 250) µmol/h · l. For exposed persons, these values ranged from 90 up to 550 (mean (porphobilinogen) = 250 ± 120) µmol/h · l erythrocytes. The activity of 5-aminolevulinate dehydratase estimated according to method II ranged from 871 to 3074 (mean = 1715 ± 590) and from 339 up to 1278 (mean (5-aminolevulinate) = 758 ± 244) µmol/h · l erythrocytes for the control and exposed groups, respectively.

Discussion

Berlin & Schaller (15) prompted analytical chemists to adopt a standard European method for the estimation of the activity of 5-aminolevulinate dehydratase in blood. These authors emphasized also that the method proposed by them is suitable for the lead concentration range up to 1.93 µmol/l blood, corresponding to the usual level of environmental exposure to this metal. The range of lead concentrations on which the method of *Tomokuni* (14) is based is equal to 0–3.38 µmol/l. *Haas* et al. (1) also used this lead concentration range.

In the present experiments, the blood lead concentration ranged from 0 to 11.60 µmol/l blood. The lower mean values of activity of 5-aminolevulinate dehydratase noted here for both the control and exposed groups, as compared with those found by *Tomokuni* (14), are due to high lead concentrations in blood. A high degree of inhibition of activity of 5-aminolevulinate dehydratase was revealed in more than 60% of the exposed persons, this activity being lowered by about 80% when estimated by the method of *Tomokuni* (14) and by only about 50% when estimated by the method of *Berlin & Schaller* (15). This difference is probably due to different pH values of the incubation media in both methods (14, 15).

Moreover, when comparing these methods, higher values of the correlation coefficient between activity of 5-aminolevulinate dehydratase activity and lead concentration in blood were found for the method of *Tomokuni* (14) (table 1). For the lead concentration range up to 4.35 µmol/l blood the value of r = 0.75 obtained here is identical with the value reported by both *Tomokuni & Ogata* (5) and *Haas* et al. (1).

These results indicate that both the method of *Tomokuni* (14) and the method of *Berlin* and *Schaller* (15) are suitable for determination of the activity of 5-aminolevulinate dehydratase in blood containing lead concentrations less than 4.35 µmol/l. The high correlation coefficient (r = 0.82) and the regression equation enable the interconversion of results between these methods.

However the ratio of activities of 5-aminolevulinate acid dehydratase determined by method I and method II (expressed in the same units) depends on the blood lead concentration (tab. 2). The variable value of this ratio is a result of the chosen pH level for incubation of this enzyme. Together with the rise of concentration of lead in blood the activity of 5-aminolevulinate dehydratase decreases at pH 6.8 (method I), and increases at pH 6.4 (method II) (14).

However, when the lead concentration in the blood of exposed persons exceeds 4.35 µmol/l, which results in a high inhibition of activity of 5-aminolevulinate dehydratase at pH 6.8, only the method of *Tomokuni* (14) can be used for the estimation of the degree of exposure to lead, based on the 5-aminolevulinate dehydratase activity ratio. A high correlation coefficient (r = 0.80) was obtained for the activity ratio and the lead concentration in blood, in the range of the latter up to 11.60 µmol/l. A rationale for this may lie in the hypothesis of *Tomokuni* (14), that lead, changing the conformation of the molecule of 5-aminolevulinate dehydratase, also alters the pH dependence of the enzyme affinity for the substrate. The maximal activity of 5-aminolevulinate dehydratase in exposed persons occurs at pH 6.0, which is taken into consideration in the calculation of the activity ratio.

The present results suggest that method I (14) is more sensitive and gives a higher correlation coefficient between concentration of lead in blood and activity of 5-aminolevulinate dehydratase than method II (15). Moreover this method is more useful for the evaluation of a broad range of lead concentrations in the blood of exposed workers.

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